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Pythium Graminicola Subr. on Barley

By WEN-CHUN HO, C. H. MEREDITH and I. E. MELHUS

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BOTANY AND PLANT PATHOLOGY SECTION

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SUMMARY

Pythium graminicola was constantly isolated from infected roots of barley grown on the experimental plots of the Northern Iowa Agricultural Experimental Farm, Kanawha, and from plants grown on the plots at the Agronomy Farm at Ames, Iowa, in 1936 to 1939, inclusive.

The symptoms of *Pythium graminicola* on barley were: Seed decay, seedling blight, root necrosis, yellowing and curling of leaves and stunting. Pythium root necrosis was very severe in 1936 and in 1938. The injury to the seedlings was greater at high temperature than at low. The pathogen grew very slowly at 15° C., which may in part explain the larger yields incident to early seedings.

Oogonia and antheridia were observed in more than 80 single zoospore cultures indicating that the pathogen is homothallic. The optimum temperature for mycelial growth of this pathogen in culture seemed to be 25° to 30° C., maximum between 35° and 40° C. and the minimum between 5° and 10° C. The pathogen was found to grow better in steamed alkaline Iowa soils such as Webster loam pH 8, than in acid soils such as Muscatine silt loam pH 5.3.

The organism penetrates the root tips in the zone just back of the cap by a small penetration peg formed from a swelling on the hyphal tip (appressorium-like). From this penetration peg subsequent invasion progresses inter- and intra-cellularly, spreads into basal portions from the epidermis through the endodermis into the central cylinder. Plants growing in glass cylinders with the pathogen placed 2 cm. above the seed showed only slight root necrosis, and the plants were somewhat stunted; while in the cylinders where the pathogen was placed below the seeds, severe root necrosis occurred.

Ten varieties of barley commonly grown in the Midwest were tested in infested, steamed soil in the greenhouse. Field trials on 8 of the 10 varieties were also conducted at the Northern Iowa Experimental Farm, Kanawha, and at the Agronomy Farm, Ames, Iowa. Data obtained from both greenhouse and field tests indicated that Ministurdi seemed to possess more resistance than any of the other varieties grown.

Pythium Graminicola Subr. on Barley[1]

By Wen-chun Ho, C. H. Meredith and I. E. Melhus

In May, 1936, it was observed that the stand of barley was unusually thin in many fields in central Iowa and that the plants were yellow and stunted. Some were flaccid and dying, and at heading time the height of the grain was very uneven. The stunted plants not only headed short but were conspicuously yellow in contrast to the dark green, healthy plants. In some cases the farmers were uncertain as to the advisability of plowing under the crop and using the land for corn. As the season advanced, however, the barley seemed to make some recovery, and a light crop of only 15.9 bushels per acre of grain was harvested. The average yield[2] of barley for Iowa in 1936 was only 15.9 bushels per acre, while in 1935 it was 26.5 bushels. The 10-year average was 23.9 bushels per acre.

The cause of this unusual condition was not apparent, and the symptoms were markedly different from any that had been observed before. This led to a study of the disease in a barley field on the Northern Iowa Experimental Farm at Kanawha, where the cropping system and soil treatment were known for the preceding 4 years. Isolations yielded chiefly *Pythium graminicola* Subr. A search of the literature on barley diseases revealed that *Pythium debaryanum* had been isolated from barley roots but no other species of Pythium. Little or no information, however, was available relating to species of Pythium attacking barley.

The object of this bulletin is to report certain studies that have been made of *P. graminicola,* its pathogenicity, the influence of environmental factors and the host response including varietal susceptibility.

PERTINENT LITERATURE

A review of literature reveals that Eriksson(6) in 1912 mentioned *Pythium debaryanum* as attacking barley and causing seedling blight. A root rot of barley caused by *P. debaryanum* was also reported in Denmark in 1921 by Gram and Rostrup(8). In 1927, Chabrolin(3) working in Tunis, North Africa, found that dead barley roots contained numerous round, thick-walled bodies that were identified by Guyot as oospores of a species of Pythium. In 1928, Subramaniam(12) described a new species of Pythium causing a root rot of wheat as *P. graminicola.* The characteristic

[1]Project 432 of the Iowa Agricultural Experiment Station.
[2]Annual report of Iowa Weather and Crop Bureau for 1936. Bul. 82, Iowa Department of Agriculture.

Fig. 1.

symptoms of the Pythium root rot of wheat given were browning, softening of the collar and blackening and splitting of the leaf-sheaths. The discoloration of the stem extended up to the first node. The underground portions of the plants also were found to be soft and blackish in color. Plants of all ages were attacked.

Vanterpool and Truscott (15) in 1932 described *Pythium arrhenomanes* var. *canadensis* as destructive to wheat, rye and corn and slightly to moderately so on oats and barley. On the other hand, they found *Pythium volutum* to be destructive on oats, wheat and rye, moderately so on barley and only slightly so on corn. The first symptoms of Pythium root rot developed during the first week in June, when the wheat seedlings were about 5 to 6 weeks old. They mentioned that under experimental conditions the pathogen caused an embryo rot, pre-emergence killing of the wheat seedling, post-emergence blight and retarded development of the host.

A more complete description of symptoms of Pythium root rot of wheat was given by Simmonds *et al.* (11). They state that the disease is characterized by yellowing and death of the lower leaves of the seedlings which gradually become unthrifty and stunted. The laterals of the seminal roots present a discolored, sickly appearance, and many of the crown roots are cut off by large lesions. The diseased plants may recover to some extent but remain more or less stunted and spindly through to maturity. The time of ripening may be delayed and the yield reduced.

Fig. 1.* Characteristic symptoms of *Pythium graminicola* in seeds and seedlings of barley, variety Velvet, grown in the greenhouse. The soil was artificially infested with the pathogen after it had been steamed. A. A healthy seed. B. A seed planted in Pythium-infested soil. Note the glumes of the seed coated with soil particles entangled in the fungous hyphae. C. Same as B with the soil particles and fungous hyphae removed. Note the disintegrated tissue of the embryo and endosperm extruding from the tip of the seed. D. Seedling blight with the radicle destroyed and the discolored young shoot. E. A seedling showing the rudimentary roots discolored and rotted. F.-G. Seedlings with all their roots discolored and rotted. Note the dark-streaked necrotic lesions on the coleoptile. H. A seedling with the infected roots partially coated with soil particles entangled in the fungous hyphae. I. A seedling with the soil particles and fungous hyphae removed, showing dark necrotic lesions on the infected roots. J. A less severely infected seedling with necrotic lesions on the young roots and basal portion of the older roots. K. The root system of a healthy seedling.

*The authors are indebted to Miss Marie A. Corkle for drawing figs. 1, 4 and 6.

Asuyama(1) described a browning root rot of wheat and barley in Japan caused by species of *Pythium* and *Nematosporangium*. More recently, Subramaniam(13) reports that *Pythium graminicola* taken from sugar cane was pathogenic on barley in India, but he failed to deal with the pathogen further than to record its pathogenicity in cross-inoculation tests. Drechsler(4), in a recent paper, illustrated and described the distinguishing characteristics of *P. graminicola* and *P. arrhenomanes*.

There are many striking points of similarity between the Pythium root rot of barley and the Pythium root rot of milo, as described by Elliot *et al.* (5). In the field the disease on milo first appeared as a slight rolling or drooping of the leaves, accompanied by retarded growth. The older leaves turned yellow and died. Subsequently the whole plant died before heading. Root symptoms were present before there were any visible symptoms on the upper part of the plant. The roots generally showed a characteristic water-soaked, brown or red discoloration of the cortex and vascular tissue.

SYMPTOMS

The symptoms of *Pythium graminicola* on barley were manifested in the greenhouse and field by seed decay, seedling blight, root necrosis and stunted growth. Although the leaves of the infected plants became yellow and dried up, there was no evidence of the pathogen on the above-ground portion of the plant. Observation and experimentation over several years have indicated that the parasitic attack of the pathogen started in the seed and very young seedlings. In the greenhouse experiments, necrotic lesions on the roots were generally noticeable when the seedlings were about a week old, but the yellowing and curling of the lower leaves did not begin to show until the seedlings were 3 or 4 weeks old.

In heavily infested soil, germination was prevented, and the seed coated with soil particles entangled in the fungous hyphae. When such Pythium-attacked seeds were washed and dropped into tubes of distilled water, the pathogen developed profusely on the surface, forming a grey, felt-like mass around the seed. In more severe cases the seed became soft, water-soaked and rotted. The pathogen seemed to attack all parts of the seed: embryo, endosperm and glumes, but the glumes remained intact. However, they were discolored, becoming dark brown instead of normal light brown (fig. 1, A, B, C) with a dark purple tint along the groove. The tissue of the embryo and endosperm was completely disintegrated. By squeezing the infected seed the disintegrated tissue consisting of a whitish grey, milky substance burst out from the tip of the seed. In greenhouse trials, seed decay was found to be more severe when the seeds were subjected to temperatures ranging from 12° to 18° C.

Badly infected germinating seeds failed to emerge from the

soil because of the rotting of the rudimentary roots and young shoots (fig. 1, D), while others, less severely attacked, were able to emerge but were yellow and stunted. The coleoptile was often attacked and showed dark yellow to reddish brown necrotic lesions (fig. 1, E, F, G). Seedlings attacked in this manner dried up and disappeared. The pathogen in the young plants caused a seedling blight.

Root necrosis caused by the pathogen might kill the seedlings or merely stunt them. When the pathogen attacked the terminal portion of the roots, the initial leaf gradually became yellow. The roots, particularly the laterals, were attacked and the lesions rapidly enlarged. The root-terminals were discolored, rotted and completely destroyed by the pathogen, so that they were torn off easily from the remaining portions when the plants were lifted or washed. The lesions on the roots varied in color from light to dark brown. The infected roots were small, from one-tenth to one-half as long as healthy roots and fewer in number. These infected roots were always partially coated with soil particles entangled in the fungous hyphae (fig. 1, H, I, J). Leaves of the severely infected plants were narrower and shorter than the leaves on healthy plants. The lower ones curled, became reddish brown and dried up.

Under field conditions the stand was reduced by seed decay. The glumes of the dead seeds were brownish black and the seed itself soft and watery. Some of the seeds that had germinated developed radicles and short shoots, but these were killed by the fungus before they emerged. Others that survived the seedling stage were yellow and much stunted. Cases also have been observed in the field in which plants infected early subsequently recovered and regained their green color but were permanently stunted.

The most striking symptom of the disease in the field was the occurrence of yellow and dwarfed plants with many of the root terminals decayed. The infected portions of the roots soon disintegrated, and only brown-tipped stubs of former lateral roots remained (fig. 2). In very severe cases the seedling toppled over, dried up and disappeared. In some cases the hypocotyl showed a brown discoloration.

As the season advanced, the stunted plants not only headed shorter but were conspicuously yellow in contrast to the green of the healthy plants. In all such cases, the many small tillers were dead. Such diseased plants were more easily pulled up than healthy ones because many of the roots were destroyed. The lower leaves died and disappeared, and the new ones tended to form rosettes (fig. 3).

The infected plants always headed short when the roots were parasitized. When serious root necrosis developed, the culms were only about one-half as tall as those of healthy plants, and the heads were very short. Infected plants seemed to stool excessively, but

Fig. 2. Characteristic root lesions caused by *Pythium graminicola*. The underground portion of this barley plant was taken from the experimental plots at Ames, Iowa, in July, 1939. Note the discoloration of the terminal portions of the roots, some are blackened and others are completely destroyed.

only a few of the stools developed culms. The culms that did head became light yellow, then brown and dried up. The grain in such seriously infected plants was generally shriveled and light and the yield seriously reduced.

CAUSAL AGENT

In June, 1936, an attempt was made to determine the causal agent of the disease already described. Isolates were taken from a field on the Experimental Farm located at Kanawha in North Central Iowa. The barley field chosen for intensive study was in corn in 1935, sugar beets in 1934 and alfalfa in 1933. The corn crop was impaired seriously by a root parasite, and the sugar beet crop was below normal because of a poor stand. Damping-off organisms seriously injured the stand. The soil where the greatest damage occurred was a Webster loam(2) having a gray calcareous subsoil. The pH range of the surface soil was 6.8 to 7.2.

The infected plant material was collected, and small portions of rootlets were cut into about 0.5 cm. lengths and washed with the aid of a camel's-hair brush to remove the attached soil particles. Material selected for isolation was placed in sterile petri dishes

with about 10 cc. of sterile, distilled water and transferred two or three times at 3-hour intervals. Surplus water was removed by placing the infected tissue between folds of sterile filter paper. The plant tissue was plated out on plain agar without added nutrients and incubated at 15° C. for the first day and 25° C. for the next day. At this time transfers were taken from the hyphal tips to other media, such as steamed carrot cubes resting on plain agar and single, steamed hemp seed dropped into 5 cc. of sterile distilled water, for identification purposes. Steamed carrot on plain agar and steamed hemp seed in water very effectively facilitated vegetative growth and sporulation.

Diseased plants were taken from the field and isolations made as noted above. On June 22, 1936, 10 typically infected plants were chosen for isolation. From all these the isolates proved to be species of Pythium of the filamentous, lobate, hyphal type. Subsequent studies seemed to indicate that seven of the isolates were *P. graminicola.* Other isolations were made from fields showing the characteristic symptoms described. For example, on May 18, 11 infected plants were taken for isolation, seven of which yielded isolates of *P. graminicola.* Two other isolates proved to be species of *Plectospira.* The tenth and eleventh plants yielded only species of *Fusaria, Rhizoctonia* and bacteria.

Fig. 3. Characteristic symptoms of older plants caused by *Pythium graminicola.* These plants were growing in the experimental plots at Ames, Iowa. The barley was seeded in May and the plants photographed July, 1939. Note the stunting, yellowing and dried lower leaves. Only one stool sent up a culm. Some of the seedlings may topple over, dry up and disappear. The terminal portions of the roots contained numerous oospores of *P. graminicola.*

Again, in the spring and summer of 1937, 41 infected plants of barley and four infected plants of *Setaria glauca* were chosen for isolation. On May 22, 8 of 15 isolates obtained from barley proved to be *P. graminicola.* On June 13, 7 of 11 isolates secured from barley yielded *P. graminicola.* Four isolates were made from *Setaria glauca* growing in the barley field; all yielded *P. graminicola.* Isolates made from barley on July 7 yielded only species of *Fusarium, Helminthosporium, Rhizoctonia* and bacteria. It was apparent that *Pythium graminicola* was more prevalent in the roots of barley in the early growing season than the late. Numerous isolations were made again in 1938 from infected plants of barley grown at Kanawha and Ames in June and July. A higher percentage of *Pythium graminicola* isolates were obtained than in 1936 and 1937.

GROWTH AND SPORULATION OF THE PATHOGEN ON STEAMED CARROTS AND HEMP SEEDS

One of the major difficulties in working with Pythiaceous root parasites is the problem of quick isolation and identification of the pathogen. Steamed carrot resting on plain agar and hemp seed in sterile, distilled water proved to be effective media for the growth and differentiation of species of *Pythium,* and particularly *P. graminicola.*

The mycelium usually started to grow 3 or 4 hours after planting, and the hyphae attained a length of .09 to .44 cm. in 5 hours at 22° C. Branching of hyphae occurred at irregular intervals at angles usually not much smaller than a right angle, and they were 2.5 to 7μ in diameter.

The oogonia began to form after 35 to 40 hours. These in most cases were borne terminally as ellipsoidal protuberances at the end of hyphal branches. The oogonia were fully formed as spherical, smooth bodies within 50 hours, varying from 29.6 to 51.8μ in diameter. In some instances oogonia in old cultures may be penetrated by hyphae which subsequently absorb their contents.

The antheridia were androgynous and diclinous one to six or more per oogonium, usually four in number and often curved measuring about 5.8μ in diameter in the distal expanded portion and 10 to 15μ in length from apex to basal septum. The antheridial filaments were usually long and irregular, occasionally two to four arising from one common hypha.

Oospores occasionally formed 2 days after the appearance of oogonia and antheridia. The spherical oospores usually did not fill the oogonium. They varied in size from 20.4 to 29.5μ in diameter, with a fairly thick wall, a central vacuole-like reserve globule surrounded by a parietal layer of granular protoplasm. Oospores became more numerous in old cultures, and the majority were 22.2μ in diameter.

Spherical bodies similar to the oogonia developed abundantly in old cultures and germinated by means of a single germ-tube. Other spherical bodies formed at low temperature (15° C.) also germinated by a single germ-tube. However, this germ-tube may be merely the continuation of growth of the inflated hypha. The sporangia of this organism developed profusely on hemp seed. They started their formation as globular intercalary, inflated elements 20 hours after planting the mycelium in the above medium.

Vesicles and zoospores of this organism may be produced readily in 20 hours by dropping 2-day-old infected seedlings into water. The vesicles were formed at the tip of long discharging tubes which extended from the filamentous, inflated elements. When young the vesicles were nearly hemispherical. Later these enlarged and became nearly spherical, 45 to 70μ in diameter. The protoplastic content of the vesicles were gradually differentiated into small globules which later escaped into the water as zoospores through an opening in the wall of the vesicles. In some cases zoospores escaped immediately, but in other cases it required several minutes. The number of zoospores per vesicle varied from 18 to 24 or more with a diameter of about 10 to 15μ. They were very active at the time of discharge but soon rounded up and came to lodge on the bottom of the plate. In 10 to 20 minutes after rounding up they developed a single germ-tube which later branched irregularly into asymmetrical filaments 2-3μ in diameter.

HOMOTHALLISM IN *PYTHIUM GRAMINICOLA*

The study of numerous isolates of Pythium obtained from barley in the past 3 years indicated that the pathogenicity and morphological characters of the causal agent were very variable. This fact suggested that it would be quite worthwhile to find out whether *P. graminicola* is homothallic or heterothallic.

In the spring of 1937, small portions of artificially infected young barley seedlings were placed in a sterile petri dish under aseptic conditions. The inoculum used to induce the infection was isolate No. 363, used in all the studies recorded in this bulletin A drop of sterile, distilled water was added to each portion of the infected barley seedlings from which Pythium hyphae emerged. The petri dishes were then incubated at 25° C. Observations were made approximately every 2 hours. As soon as zoospores were observed, a very fine, glass pipette was applied to draw out the water which contained many zoospores. This was spread on the surface of a plain agar medium. Hyphal tips were cut from the germinating zoospores and transferred to potato-dextrose agar slants. A large number of single-spore cultures of *P. graminicola* were obtained by this procedure, of which 49 were observed critically for the appearance of sex organs by growing them on plain agar with steamed carrot. None of the 49 cultures developed

only antheridia or oogonia. Repeated microscopical examination showed that these single zoospore cultures were all homothallic, like the parent culture inoculated into the barley seedling in the beginning. The above experiment was repeated in the fall of 1937 and in the winter of 1938. In 1938, 38 single-spore cultures were examined for the presence of sex organs. None of the 38 cultures developed only antheridia or oogonia. Both sex organs were always present, and the organism was homothallic.

It is of special interest that recently, Vanterpool(14) has demonstrated that five species of Pythium, including the diclinous *P. arrhenomanes* are all homothallic. The work described above was done before Vanterpool published his work, indicating that the homothallic nature of these species of Pythium is rather certain.

EFFECT OF TEMPERATURE AND SOIL REACTION ON THE DEVELOPMENT OF *PYTHIUM GRAMINCOLA* SUBR.

It already has been noted that as the season advanced the barley crop seemed to recover. This observation raised the question as to the influence of temperature on the growth and development of the pathogen.

Vanterpool and Truscott(15) while working with *Pythium arrhenomanes* var. *canadensis* on wheat reported that the optimum temperature for vegetative growth of the organism ranged from 30° to 35° C. Its actual damage to wheat seedlings was found to be least at 24° C. and increased at lower (12° C.) and higher temperatures (31° C.). The amount of injury also increased with the amount of moisture in the soil.

Johann *et al.* (10) found that the growth of *Pythium arrhenomanes* taken from maize on potato-dextrose agar grew between 4° and 40° C. and that the optimum temperature from vegetative growth was 30° to 36° C. Sporangia appeared profusely at temperatures ranging from 20° to 30° C., but the formation of oospores was favored by storage at lower temperatures (10° to 16° C.). Their experiments showed that soil temperatures near 16° C. or lower, together with high soil moisture, were favorable for infection.

Flor(7) found that the growth rate of Pythium, isolated from the roots of sugar cane, in Difco dextrose agar increased with temperature to 30° C. and fell off sharply at 36° C. Corn was not injured at 35° C., but severe injury was obtained as the temperature was lowered. Injury also became greater with an increase in water content of the soil.

The effect of temperature on the growth of *Pythium graminicola* was measured by placing the organism in soil and on plain

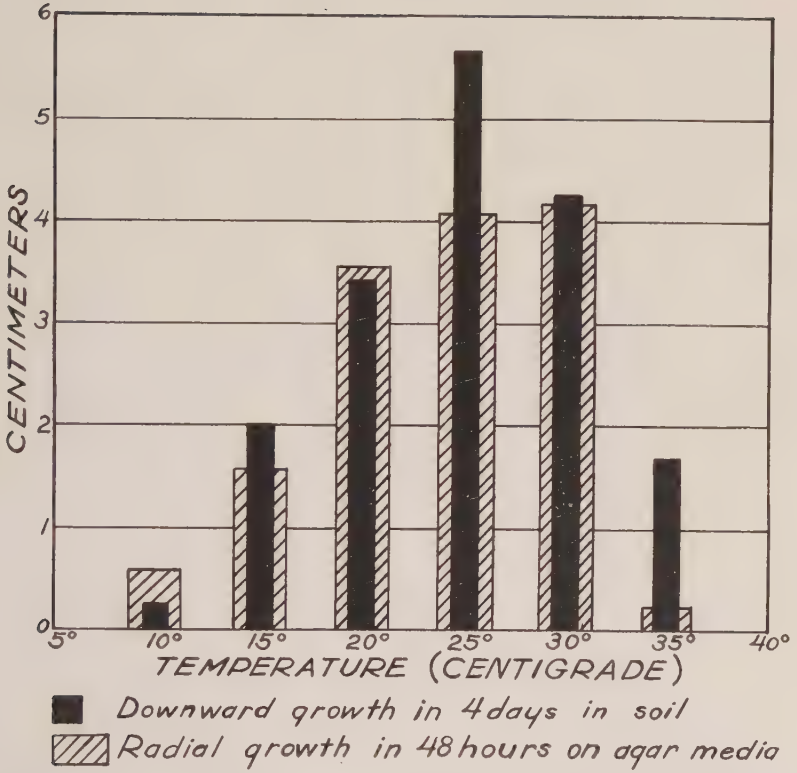

Fig. 4. The mycelial growth of *Pythium graminicola* at various temperature conditions.

agar incubated at various temperatures ranging from 0° to 40° C. at 5-degree intervals. Three experiments on the effect of temperature were made and are summarized in fig. 4. The soil used in these studies was taken from the infested barley field already described, air dried and placed in large test tubes, 1.5 cm. in diameter and 15 cm. in length. Soil in the tubes was moistened and steamed for 1 hour at 15 pounds pressure. After cooling, a fragment of plain agar carrying *Pythium graminicola* was placed on the surface of the soil in each tube and held at temperatures ranging from 0° to 40° C. Data in fig. 4 show the optimum temperature near 25° C. and the minimum between 5° and 10° C. when the organism is grown in soil.

The temperature relations were studied still further in another experiment using three separate trials of duplicate plate cultures containing small cubes of steamed carrot resting on plain agar. Fragments as nearly equal in size as possible of a 2-day-old agar culture of *P. graminicola* were transferred to the edge

of the carrot and incubated at different temperatures ranging from 0° to 40° C. Data in fig. 4 indicate that the mycelium did not grow at 5° C., but that it grew slowly at 10° C., moderately at 15° C. and rapidly at 20°, 25° and 30° C. Mycelial growth was very meager at 35° C. Throughout all of the three trials the optimum temperature for mycelial growth seemed to be between 25° and 30° C. (fig. 4). Oogonia and antheridia formed most rapidly and abundantly at 30° C. So far no formation of oogonia and antheridia was observed to develop at 35° C. or below 15° C. during the first 72 hours. The fact that *P. graminicola* grew so slowly below 15° C. may in part explain the larger yields of early seedings of barley in Iowa. Late-sown barley seldom yields well. The higher temperatures of late May and early June are more favorable for *P. graminicola* than those in late March and early April. Since the season of 1936 was unusually hot and dry, it is conceivable that in late June and early July the soil temperature was above the optimum for the most rapid growth of *Pythium graminicola,* and the diseased plants seemed to make considerable recovery in the late season.

Many cultural studies on artificial media indicated that *P. graminicola* grew better in an alkaline medium than in an acid one. In order to learn whether this same response to soil reaction occurred in soil, samples of soils with different reactions collected from various parts of Iowa were used to grow the pathogen. The various soil samples with suitable moisture were placed in large glass tubes. The reaction of each soil sample was determined after it had been steamed in autoclave for 1 hour at 15 pounds pressure. After the tubes had cooled a fragment of plain agar carrying the pathogen was placed on the surface of the soil in each tube, and the tubes were held at 25° C. Measurements of the mycelial growth were taken daily and were ended on the seventh day, at which time the difference of mycelial growth in all soil samples was distinctly shown. The experiment was repeated four times, and the data obtained consistently confirmed that the

TABLE 1. THE DEVELOPMENT OF *PYTHIUM GRAMINICOLA* ON SOILS OF VARIOUS TYPES AND pH VALUES IN GLASS TUBES HELD AT 25° C.

Soil type	Source of soil	pH of soil*	Downward growth in 7 days
			cm.
Webster loam	Kanawha	8.0	6.0
Webster silty clay loam	Ames	7.6	5.5
Wabash silty clay loam	Ames	6.7	4.8
Clarion loam	Ames	6.0	2.7
Muscatine silt loam	State Center	5.3	0.2
Tama silt loam	State Center	5.2	0.3

*Average readings of four determinations.

growth of *P. graminicola* in neutral to slightly alkaline soils was decidedly better than in acid soils (9). Webster loam obtained from the Northern Iowa Experimental Farm having a pH 8 gave the best mycelial growth of the organism, while the Muscatine silt loam having a pH 5.3, obtained from State Center, Iowa, permitted very little growth (table 1).

PATHOGENICITY OF *PYTHIUM GRAMINICOLA* SUBR. ON BARLEY

PENETRATION

Penetration was studied in free-hand sections of the roots which were infected by placing barley seedlings with Pythium growing in petri dishes on plain agar (fig. 5). The pathogen penetrated the epidermal cells of the roots within 2 hours. It was found that the organism readily penetrated and invaded the meristematic tissue in the root-tip area, especially the lower part of the root-hair region. The root-hairs were not attacked as frequently as the adjoining epidermal cells. The penetration of the basal portion of a root-hair was observed. Where the infection hypha came in contact with either the calyptrogen of the root-cap (fig. 6A) or the epidermis of the root-hair region, (fig. 6B) a swelling (appressorium-like) developed at the hyphal tip from which a small penetration peg was formed. This penetration peg pierced the cell wall and, once inside, assumed the normal size of the mycelium.

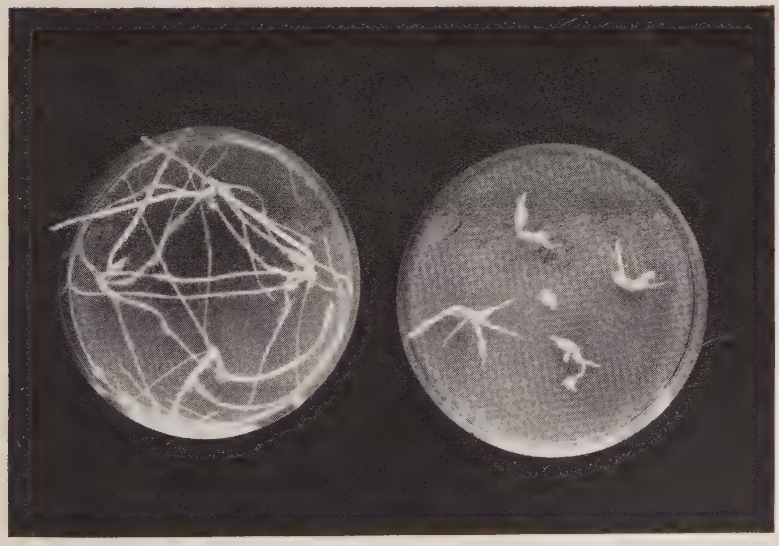

Fig. 5. Barley seedlings on a plain agar plate (left) and on a plain agar culture of *P. graminicola* (right).

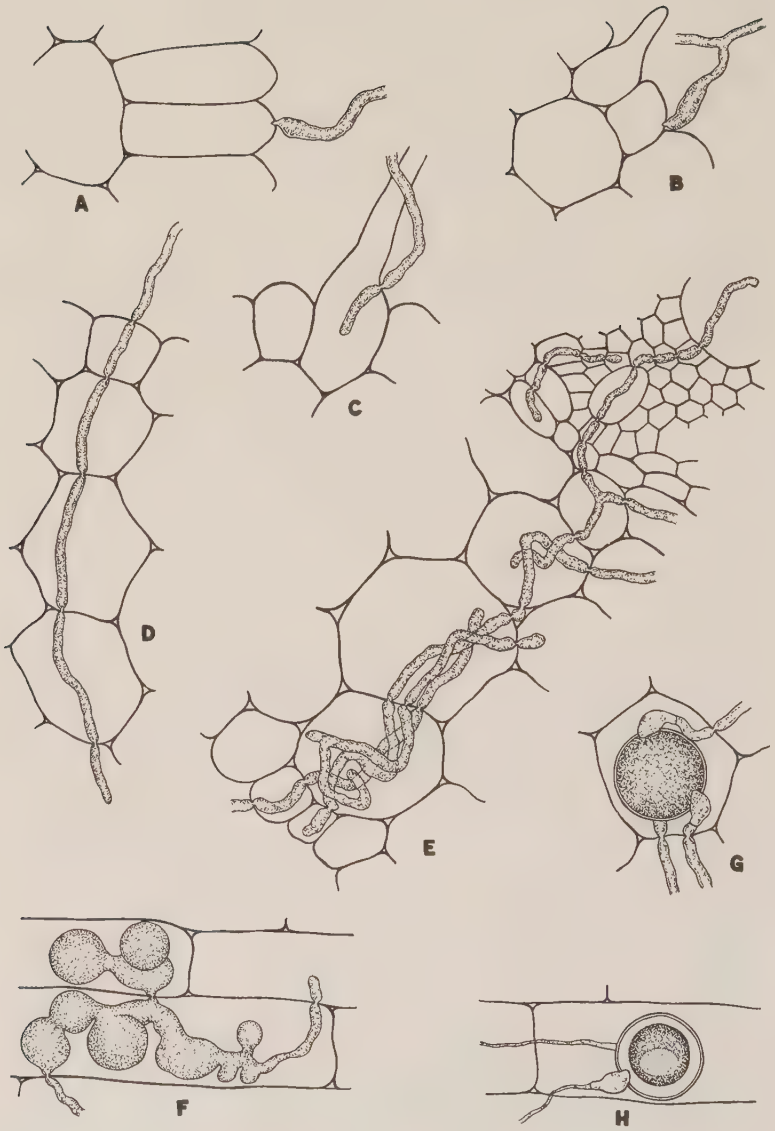

Fig. 6.

There was no dissolution of the cell wall. The penetration seemed to be accomplished chiefly by mechanical pressure of the growing mycelium as shown in fig. 6C.

The hypha in the epidermal cell, when it came in contact with a wall, again formed a penetration peg which passed through (fig. 6D). Subsequent invasion progressed very rapidly throughout all the parenchymous tissue. It was observed that it required 6 hours for the pathogen to invade the root and penetrate into the xylem. Upon reaching the endodermis, the hyphae penetrated less readily. The growth of the hyphae from the epidermis through the parenchyma and endodermis into the fibro-vascular bundle is shown in fig. 6E.

Filamentous sporangia developed abundantly in the parenchyma 24 hours after infection as illustrated in fig. 6F. Approximately 48 hours after penetration, the young oogonia of the pathogen were more abundant in the stele than in the parenchyma tissue. Oogonia with antheridia (fig. 6G) appeared in 3 to 4 days, and oospores were abundant in 9 (fig. 6H).

EFFECT OF TEMPERATURE ON PATHOGENICITY

The development of seedling injury was studied in the winter of 1936 by growing seedlings in steamed soil in glass tubes as shown in fig. 7. These tubes were 3.0 cm. in diameter and 20.0 cm. in length, plugged with cotton in each end. Healthy seeds of barley were selected and washed with distilled water for 30 minutes and then rinsed in sterile, distilled water twice before planting. Six seeds were planted in each tube. The culture

Fig. 6. A. *Pythium graminicola* invading a root-cap cell by the development of a penetration peg. This stage of penetration occurred within 3 hours after the inoculum was placed in contact with the root. B. A hypha penetrating the epidermis of the root in the root-hair region. C. In this case a hypha has penetrated a root-hair cell. Note the restriction of the hypha at the cell wall of the host. The extent of penetration occurred within 4 hours. D. Development of single hypha in the epidermal and parenchyma cells of a root occurred in 6 hours. E. The growth and development of hyphae in the parenchyma, endodermis and fibrovascular bundle. The pathogen was inter- and intra-cellular. This development required only 8 hours. F. Filamentous sporangia appeared in the parenchyma cells within 24 hours. G. An oogonium and two antheridia appeared in the parenchyma cell of a root 64 hours after it was exposed to infection. H. An oospore with one antheridium still attached to the oogonial wall in a parenchyma cell of a root.

of the organism was placed 2 cm. above the seed in three tubes and the same distance below in three others with steamed soil separating the seed from the organism. The three checks contained seed planted without the organism and covered with 2 cm. of steamed soil.

TABLE 2. TOP GROWTH AND MAXIMUM ROOT GROWTH (CM.) OF BARLEY SEEDLINGS WHEN *PYTHIUM GRAMINICOLA* WAS PLACED ABOVE AND BELOW THE SEED.

	Top growth								
Seedling No.	Check			Pathogen above			Pathogen below		
1	10.1	10.5	9.3	5.0	8.4	8.1	6.4	3.4	2.8
2	9.6	10.2	9.3	4.7	8.1	7.4	2.5	3.1	2.8
3	9.6	10.2	8.3	4.6	6.6	7.3	2.2	2.7	1.1
4	9.0	10.1	7.6	4.3	5.5	5.4	2.1	2.0	0.9
5	8.8	10.0	7.6	4.2	4.4	2.7	2.0	1.7	0.7
6	8.1	.....	5.0	3.0	4.1	.....	1.3	0.6	.....
Sum	55.2	51.0	47.1	25.8	37.1	30.9	16.5	13.5	8.3
No. seeds	6	5	6	6	6	5	6	6	5
Mean top growth	9.2	10.20	7.85	4.30	6.18	6.18	2.75	2 25	1.66
Max. root length	13.90	13.20	13.40	12.20	11.20	13.00	4.70	4.20	2.9

Analysis of Variance

Sources of variation	Degrees of freedom	Mean square
Total	50	.....
Between treatment	2	194.550**
Between tubes with same treatment	6	5.896
Plants in same tube	42	1.838

TABLE 3. THE RELATION OF TEMPERATURE TO THE PATHOGENICITY OF *PYTHIUM GRAMINICOLA* ON BARLEY SEEDLINGS IN THE SOIL AFTER 7 DAYS.

Temperature*	Inoculum	Average height seedlings	Average length roots	Average radial spread Pythium
		cm.	cm.	cm.
10° C. for 9 days	Check (none)...	1.40	7.94	....
	Above seed.....	2.05	8.04	2.61
	Below seed.....	1.66	2.92	2.90
15° C. for 8 days.....	Check (none)...	5.12	10.06	....
	Above seed.....	5.10	9.94	2.94
	Below seed.....	3.65	2.90	3.97
20° C. for 7 days.....	Check (none)...	12.69	12.30	....
	Above seed.....	7.74	10.31	5.73
	Below seed.....	3.00	3.10	7.17
25° C. for 6 days.....	Check (none)...	12.55	11.96	....
	Above seed.....	8.31	8.73	6.73
	Below seed.....	2.88	4.13	7.47
30° C. for 6 days.....	Check (none)...	11.28	10.71	....
	Above seed.....	4.10	7.76	8.52
	Below seed.....	2.47	3.98	6.94

*When the plants were from 1 to 3 cm. high they were placed in sunlight at temperatures ranging from 22° to 27° C.

The glass-tube cultures were held at 10° C. for 7 days in the dark and then 5 days in sunlight at 22° to 27° C. At the end of 7 days some of the plants were 1 cm. above the soil surface. The glass tubes permitted an examination of the roots and the pathogen with a binocular. The initial root injury appeared within 5 mm. of the root-tip, but later the entire root decayed. There was no apparent injury to the root-hairs as long as the growing point was not infected. The data obtained in these trials are shown in table 2. The differences among the treatments are highly significant.

The cultures with the organism 2 cm. above the seed at planting made about the same root growth as the check plants, but some of the leaves were shorter and turned yellow and wilted; others were a darker green than the check. The barley plants with the organism below the seeds were injured most. No healthy roots were observed below the region of the inoculum, and rapid necrosis of the whole root system was evident. The leaves in most cases did not unroll and turned brown and dried up in a few days.

Table 3 presents a summary of three separate experiments consisting of three glass tube cultures at five different temperatures, 10° to 30° C. at 5° intervals. Each experiment comprising nine cultures at each of five temperatures was treated as follows: Three were held as checks, in three the pathogen was placed above the seeds and in the other three the pathogen was laid below the seeds. The greatest injury to the seedling as a whole occurred when the inoculum was placed below the seed. In only two cases out of 180 did the root grow through the inoculum. At 20° C. or above in the tubes where the inoculum was placed above the seed the growth of the seedling was retarded. The average radial spread of the pathogen increased rather uniformly with temperature.

The difference in the height of the seedlings in the noninfested soil tube and the infested soil tubes was indicative of the injury induced by *P. graminicola* to the roots and is illustrated in fig. 7.

VARIETAL RESISTANCE AND SUSCEPTIBILITY[3]

Experiments on varietal resistance and susceptibility of barley to *Pythium graminicola* were conducted in greenhouse flats at four different times during the spring and fall of 1937. At each date, 15 seeds of each of the following 10 varieties were planted: Spartan, Peatland, Trebi, Manchuria, Velvet, Wisconsin 5, Wisconsin 38, Glabron, Ioglas and Ministurdi. The soil was prepared by mixing 1 part of sand with 3 parts of loam, and then

[3]The authors wish to acknowledge the helpful suggestions given by Professor G. W. Snedecor, director of the Statistical Laboratory, Iowa State College, in connection with the statistical phases of this paper.

Fig. 7.

both soil and flats were steamed for 6 hours at 20 pounds pressure. In one series of flats the steamed soil was infested with culture of *P. graminicola* growing on plain agar at the rate of one plate culture per flat. Three-day-old culture of the organism was divided into small cubes, mixed with an equal amount of steamed soil and distributed into rows across the flat. The inoculum used in all these experiments was isolate 363, obtained from the infected root of barley in the summer of 1936 at the Northern Iowa Experimental Farm, Kanawha, Iowa. Flats of uninfested steamed soil were used as checks.

The percentage of germination, the top growth and amount of root injury of each of the 10 varieties were noted. Observations showed that there was wide variation in the resistance of the different varieties and that resistance manifested itself in various forms.

TABLE 4. AVERAGE NUMBER OF SEEDS GERMINATING, AND PERCENTAGE DECREASE OF GERMINATION OF BARLEY SEED CAUSED BY *PYTHIUM GRAMINICOLA*.
(Average number of seeds planted, 15.)

Variety	Check	Soil infested	Percentage decrease*
Spartan	7.778	6.778	.1285
Trebi	15.000	14.500	.0345
Velvet	15.000	8.250	.4500
Wisconsin 5	15.000	14.000	.0666
Wisconsin 38	15.000	13.778	.0814
Peatland	14.778	9.778	.3383
Glabron	15.000	15.000	.0000
Ioglas	15.000	15.000	.0000
Ministurdi	8.778	7.778	.1139
Manchuria	15.000	14.778	.0148

Analysis of Variance of Transformed Values

Sources of variation	Degrees of freedom	Mean square	F	1% point
Total	39	.....	.....	...
Variety	9	.03814**	19.71	3.14
Series	3	.00025	.....	....
Error	27	.00194	.....	...

*Transformed value = $\sqrt{\text{Percentage decrease}} + .5$

Fig. 7. Seedling blight and root necrosis of barley caused by *Pythium graminicola* held at 10° C. for the first 7 days, and 5 days at 22° to 27° C. From left to right: check; pathogen placed above the seeds; pathogen placed below the seeds.

Data in table 4 summarize the percentage decrease of germination of seeds caused by the pathogen. In order to test the significance of the differences among different varieties, a transformed value as shown at the bottom of table 4 was used for the analysis of variance. The percentage decrease of germination of varieties such as Velvet and Peatland was great; was moderate in Wisconsin 5, Wisconsin 38, Ministurdi and Spartan; and slight or none in Glabron, Ioglas, Manchuria and Trebi. The differences among these groups of varieties based on their percentage decrease of germination are significant.

Table 5 presents the percentage decrease of top growth of the seedlings of different varieties caused by the pathogen. These varieties can be easily assorted into three groups based on the percentage decrease of their top growth. Ministurdi, Glabron and Ioglas showed little decrease; Manchuria, Velvet, Wisconsin 5 and Peatland a considerable decrease; and Trebi, Wisconsin 38 and Spartan a heavy decrease. As indicated by the analysis of variance, the difference in percentage decrease of top growth of the seedlings among different varieties is highly significant.

A comparison of the data in tables 4 and 5 reveals that there is no definite correlation between the percentage decrease of seed germination and the percentage decrease of top growth among different varieties, although they were both caused by the pathogen.

TABLE 5. AVERAGE AMOUNT (CM.) AND PERCENTAGE DECREASE OF TOP GROWTH OF BARLEY SEEDLINGS CAUSED BY *PYTHIUM GRAMINICOLA*.

Variety	Check	Soil infested	Percentage decrease
Spartan	24.750	16.650	.3272
Trebi	27.500	21.000	.2363
Velvet	24.625	20.175	.1807
Wisconsin 5	25.037	20.615	.1766
Wisconsin 38	25.125	19.325	.2308
Peatland	25.125	21.275	.1532
Glabron	27.750	25.100	.0954
Ioglas	27.625	26.372	.0453
Ministurdi	24.825	23.900	.0372
Manchuria	25.875	22.315	.1375

Analysis of Variance

Sources of variation	Degrees of freedom	Mean square	F	1% point	Least difference for significance
Total	39	.....	.....	.....	....
Variety	9	.03468**	41.77	3.14	....
Series	3	.00275	.....	....	....
Error	27	.00083	.....	....	.041

Table 6 shows the percentage decrease of root length of the seedlings of different varieties caused by the pathogen. These varieties may be arbitrarily divided into five groups according to their percentage decreases: Very slight, Ministurdi; slight, Glabron and Ioglas; moderate, Manchuria; heavy, Spartan, Velvet and Wisconsin 5; and very heavy, Wisconsin 38, Peatland and Trebi. As shown in the analysis of variance, the difference in the percentage decrease of root length of the seedlings of different varieties is significant.

There was a tendency for varieties showing heavy decrease of top growth to show also heavy or very heavy decrease of root length as a result of the infection of the pathogen. However, the variation in percentage decrease of root length is considerably smaller than that in percentage decrease of top growth.

Eight varieties that were used in the greenhouse experiments were planted in three 5-foot rows on the experimental plots where the soil was consistently infested with *Pythium graminicola* at the Northern Iowa Experimental Farm, Kanawha, Iowa. The first planting was made on April 20 and the second on May 16, 1937. The temperature was slightly above normal, and the soil was wet throughout the early part of the growing season. Sugar beets had been grown on these plots in 1936.

In 1938, these same eight varieties were again planted at Kanawha on April 15 and May 14 and at Ames on April 13. The previous crop at Kanawha was sugar beets, and at Ames it was oats. The temperature was low, and the soil was wet throughout the early part of the growing season.

TABLE 6. AVERAGE AMOUNT (CM.) AND PERCENTAGE DECREASE OF ROOT LENGTH OF BARLEY SEEDLINGS CAUSED BY *PYTHIUM GRAMINICOLA*.

Variety	Check	Soil infested	Percentage decrease
Spartan	8.375	5.078	.3939
Trebi	12.575	7.000	.4433
Velvet	11.375	7.125	.3736
Wisconsin 5	12.000	7.225	.3979
Wisconsin 38	11.875	6.750	.4315
Peatland	9.875	5.575	.4354
Glabron	9.250	7.250	.2162
Ioglas	10.750	8.400	.2186
Ministurdi	7.875	6.750	.1428
Manchuria	10.750	7.625	.2906

Analysis of Variance

Sources of variation	Degrees of freedom	Mean square	F	1% point	Least difference for significance
Total	39	.....	....	....	....
Variety	9	.0481**	5.88	3.14	....
Series	3	.0149	....	....	....
Error	27	.0082	....	....	.13

TABLE 7. YIELDS (IN BUSHELS) OF EIGHT VARIETIES OF BARLEY GROWN ON THE PYTHIUM-INFESTED EXPERIMENTAL PLOTS AT KANAWHA, IOWA, IN 1937 AND 1938.

Variety	Season		Sum	Mean
	1937	1938		
Ministurdi	30.60	34.80	65.40	32.70
Wisconsin 5	25.48	25.77	51.25	25.62
Spartan	24.50	25.00	49.50	24.75
Glabron	24.00	23.20	47.20	23.60
Manchuria	22.50	23.70	46.20	23.10
Peatland	20.80	24.00	44.80	22.40
Velvet	19.40	21.50	40.90	20.45
Trebi	17.60	18.20	35.80	17.90
Sum	184.88	196.17	381.05	.....
Mean	23.11	24.52	.....	23.815

Analysis of Variance

Sources of variation	Degrees of freedom	Mean square	F	1% point	Least difference for significance
Total	15	.....	....	....	....
Variety	7	37.70**	27.6	7.0	....
Season	1	7.97	....	....	....
Error	7	1.37	....	....	2.76

Data in table 7 summarize the average yield from two plantings of the eight varieties grown at Kanawha, Iowa, in 1937 and 1938. The differences in yield among these eight varieties as indicated by the analysis of variance is highly significant.

The data obtained from the field experiments and those obtained from the greenhouse experiments indicate that the variety Ministurdi not only germinated well but that its top growth and root length showed little decrease and that it was the highest yielder observed in the field experiment. In Peatland and Velvet, the decrease of seed germination as well as the decrease of root length was heavy, while the decrease of top growth was moderate. Their yields were comparatively low. Though the decrease of germination of Trebi was only slight, the decrease of its top growth and root length was rather heavy. It was the lowest yielder found among all the varieties tested in the field. The yields of Wisconsin 5, Spartan, Glabron and Manchuria were all intermediate, but their response to *Pythium graminicola* as manifested in the greenhouse experiments varied considerably.

The root injury caused by the pathogen seemed to be more effective in reducing the yield than the decrease in top growth. (See tables 5 and 6.) The decrease in germination in top growth, in root length or in a combination of these resulted in some reduction of yield. The different yields obtained from the field experiment corresponded, in general, to the response of these

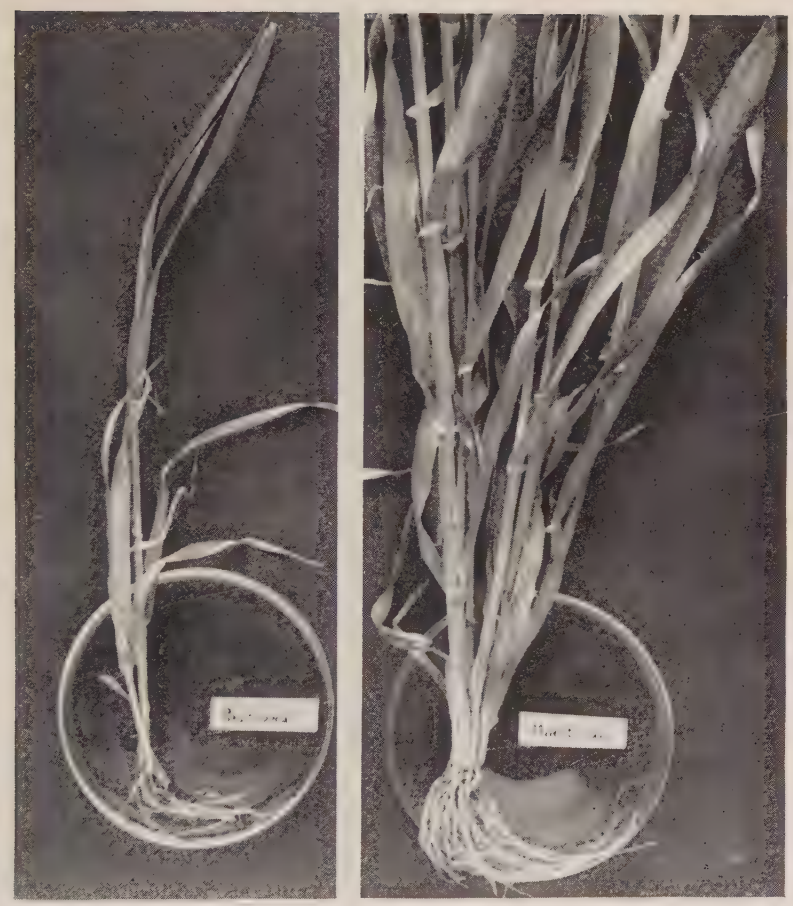

Fig. 8. The effect of *Pythium graminicola* on two varieties of barley (Peatland, left; Ministurdi, right) under field conditions. These plants were taken from the barley nursery at the Agronomy Farm, Ames, Iowa, in 1938. Note the difference in size and the number of roots on the two plants. The root system of Peatland was seriously parasitized by *Pythium graminicola*.

eight varieties to the pathogen observed in the greenhouse experiments. The severe root injury caused by the pathogen may explain, in part, why a high-yielding variety such as Trebi suffered a heavy reduction in yield.

The difference in root systems of two plants is shown in fig. 8, one of the susceptible Peatland, the other of the resistant Ministurdi, grown on the experimental plots at Ames, Iowa, in 1938. Not only was there a marked difference in the height of the plants but also in the color and number of yellow and partially dead leaves. In Peatland the roots were seriously damaged, but in Ministurdi the injury was not as serious as in Peatland.

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